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SEM Studies on Vessels in Ferns. 13. *Nephrolepis*

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ABSTRACT.—Vessels are present in roots, rhizomes, and stolons of *N. exaltata*; vessel elements are probably present, but little different from tracheids, in the tubers of *N. cordifolia*. This contrast correlates with putatively slower conductive rates in an organ that stores water. The vessels have perforation plates both on end walls and lateral walls. Both types of perforation plates are similar to lateral walls except for absence of pit membranes. Perforation plates comprise a large area of vessel surfaces; this characteristic has been observed in other ferns from habitats in which marked fluctuation of water availability occurs. As shown in other papers of this series as well as the present paper, adaptation to ecological conditions is more important than phylogenetic position in explaining the presence and degree of specialization of vessel elements in ferns.

Nephrolepis exaltata (L.) Schott is a fern that has been described as epiphytic or epipetric (Lellinger, 1985); our specimen was collected in crevices of recent lava flows in the Hawaiian Islands. The species extends from central Florida into tropical Central and South America and tropical portions of the Pacific and the Old World (Hillebrand, 1888; Lellinger, 1985; Tryon and Tryon, 1982). Thus, *N. exaltata* is a tropical and subtropical species that occupies exposed and pioneering habitats within its range, although it is also found in shady and moist localities, as in the Hawaiian Islands (Neal, 1965). The pioneering characteristics of *N. exaltata* and its ability to withstand full sun and periodic drought are features of interest with respect to morphology of tracheary elements. In at least some ferns of dry localities, vessel elements with more specialized perforation plates occur, as in *Pteridium* (Carlquist and Schneider, 1997a), *Astrolepis* (Carlquist and Schneider, 1997b), *Woodsia scopulina* D.C. Eaton (Schneider and Carlquist, 1998a), and *Woodsia ilvensis* (L.) R. Br. (Carlquist and Schneider, 1998a), whereas in ferns of moist localities, vessels may be present, but perforation plates are like lateral wall pitting except for absence of pit membranes (e.g., Osmundaceae and Schizaeaceae; Carlquist and Schneider, 1998b).

The habit of *Nephrolepis* offers distinctive organs that invite study with respect to potential diversity in morphology of tracheary elements. In addition to presence of relatively thick rhizomes, *Nephrolepis exaltata* plants bear relatively slender stolons. Tubers are formed on the slender stolons of *N. cordifolia* (L.) C. Presl. Because flow rates might be expected to be slower in the tubers because they function in storage of water and probably other substances, one might not expect perforation plates adapted to promoting rapid conduction rates in the tubers.

Our studies have concentrated on ferns from habitats that show pronounced fluctuation of temperature and water availability, such as *Polystichum* from areas that freeze in winter and *Phlebodium*, a tropical epiphyte (Schneider and Carlquist, 1997). Nevertheless, our choices have also been made with the aim of surveying a diversity of ferns with respect to systematic position. *Nephrolepis* belongs to a family we have not studied previously, Davalliaceae, although recently it has been placed in a monotypic family, Nephrolepidaceae (see Tryon and Tryon, 1982, who place it in Davalliaceae but with some reservations).

MATERIALS AND METHODS

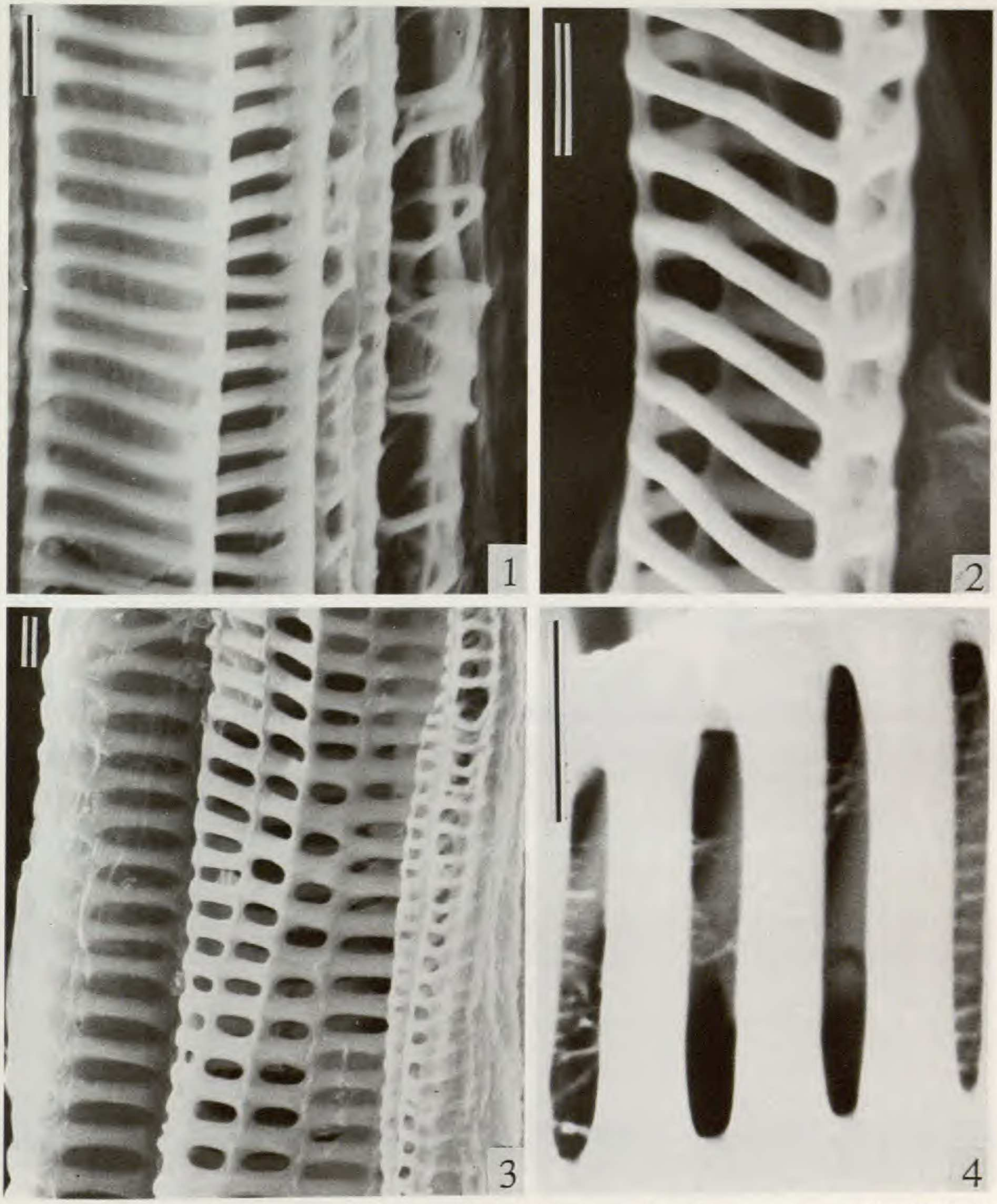
Roots, rhizomes, and stolons of *N. exaltata* were collected on a lava flow at the 92.5 mile marker on Highway 11, near Kipahoehoe Natural Area Reserve, on the island of Hawaii, Hawaii, September, 1997. Tubers of *N. cordifolia* (L.) Presl were obtained from plants cultivated at the Ganna Walska Lotusland Foundation, Santa Barbara, California. Portions were preserved in 50% aqueous ethanol.

Macerations of vascular tissue from roots, rhizomes, stolons, and tubers were prepared using Jeffrey's Fluid and stored in 50% aqueous ethanol. Macerations were spread onto the surfaces of aluminum stubs, air dried, sputter-coated, and examined with a Bausch and Lomb Nanolab scanning electron microscope (SEM). Our earlier studies (e.g., Carlquist and Schneider 1997a) showed that macerations were as reliable as sections in preserving pit membranes. Pit membrane removal due to processing is evident in the form of tearing of membranes and membrane remnants at the edges of pits, and the pattern of complete absence of pit membranes on areas corresponding to perforation plates (i.e., end walls of tracheary elements) in other vascular plants confirms this interpretation.

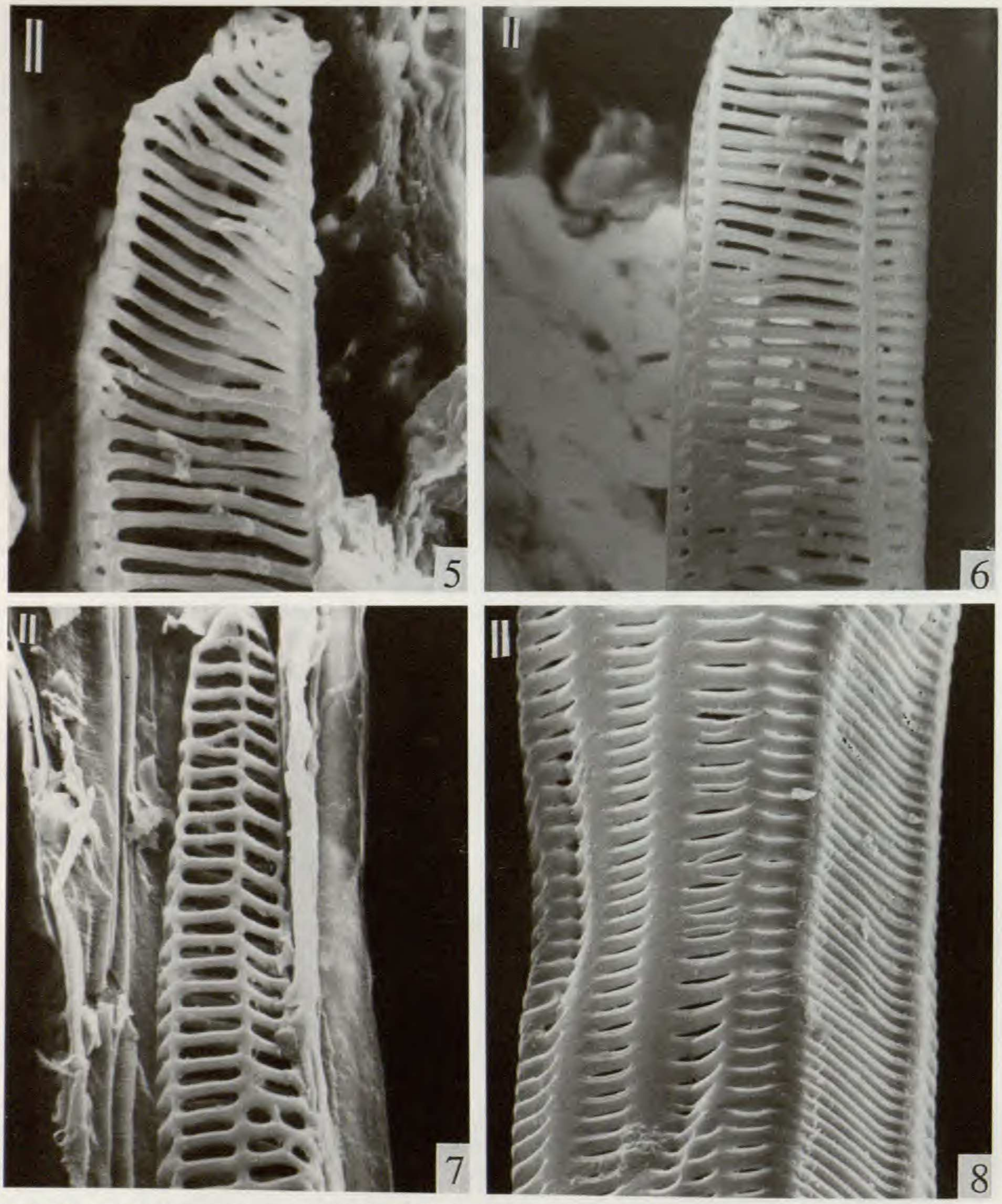
RESULTS

In tracheary elements of roots (Figs. 1–4), facets that lack pit membranes and are therefore perforation plates are common. These perforation plates resemble lateral wall pitting in all respects but pit membrane presence (a lateral wall is shown at left in Fig. 1 and at left in Fig. 3). In Fig. 2, a portion of a tracheary element in which three of the four facets are perforation plates is shown (two of these are on the back side of the cell); the narrow cell facet, right, contains pit membranes. Likewise, several adjacent facets that are perforation plates are present in the wide vessel at center in Fig. 3. Pit membrane remnants can be seen in some perforations at ends of perforation plates. In Fig. 4, the perforation at left contains threadlike remnants of the pit membrane; the two perforations, center, have small portions of weblike pit membranes; and the pit at right contains a striate pit membrane that is nearly intact.

In rhizomes, long perforation plates with numerous bars, much like lateral wall pitting except for absence of pit membranes, are present (Figs. 5, 6). The vessel element shown in Fig. 5 shows a cell tip that bears a perforation plate, only a small portion of which is shown. The cell facets of the vessel element shown in Fig. 6 are probably mostly lateral walls (we were not able to delineate



FIGS. 1–4. Portions of tracheary elements of *Nephrolepis exaltata* from root macerations. 1) Adjacent tracheary elements showing (extreme left), lateral wall pitting in metaxylem element; perforation plate in metaxylem element; and, at right, protoxylem elements. 2) Tracheary element in which facing wall and two walls on the back side are perforation plates; facet at right bears lateral wall pitting. 3). Adjacent tracheary elements showing (extreme left) lateral wall pitting, and (central element) several facets bearing perforation plates. 4) Portion of perforation plate showing strandlike pit membrane remnants (left), weblike remnants (center two perforations), and nearly intact pit membrane (right). Scale bars in all figs. = 5 μm.



FIGS. 5–8. Tracheary elements of *Nephrolepis exaltata* from rhizome macerations (5–6) and stolon macerations (7–8). 5) Tip of element, showing portion of a perforation plates. 6) Wide tracheary element; all lateral walls except that at extreme right are clear of pit membranes and qualify as perforation plates. 7) Tip of element; the facets shown are portions of perforation plates. 8) Central portion of wide tracheary element; although slits are present in some pits, these may be artifacts, and all of the facets shown appear to bear lateral wall pitting. Scale bars in all figs. = 5 μm.

end walls in this particular cell, however). Because debris behind the cell may clearly be seen through the cell, pit membranes are absent on most of the facets (at least some pit membranes are present on the facet at right). Thus, lateral wall perforation plates are present.

Tracheary elements of a stolon (Figs. 7, 8) show a range of expressions. End-wall perforation plates are present on some vessel elements (Fig. 7). The very wide tracheary element in Fig. 8 has numerous facets. All of these facets have lateral wall pitting; some pits have slitlike gaps in their membranes, which we interpret as probable artifacts.

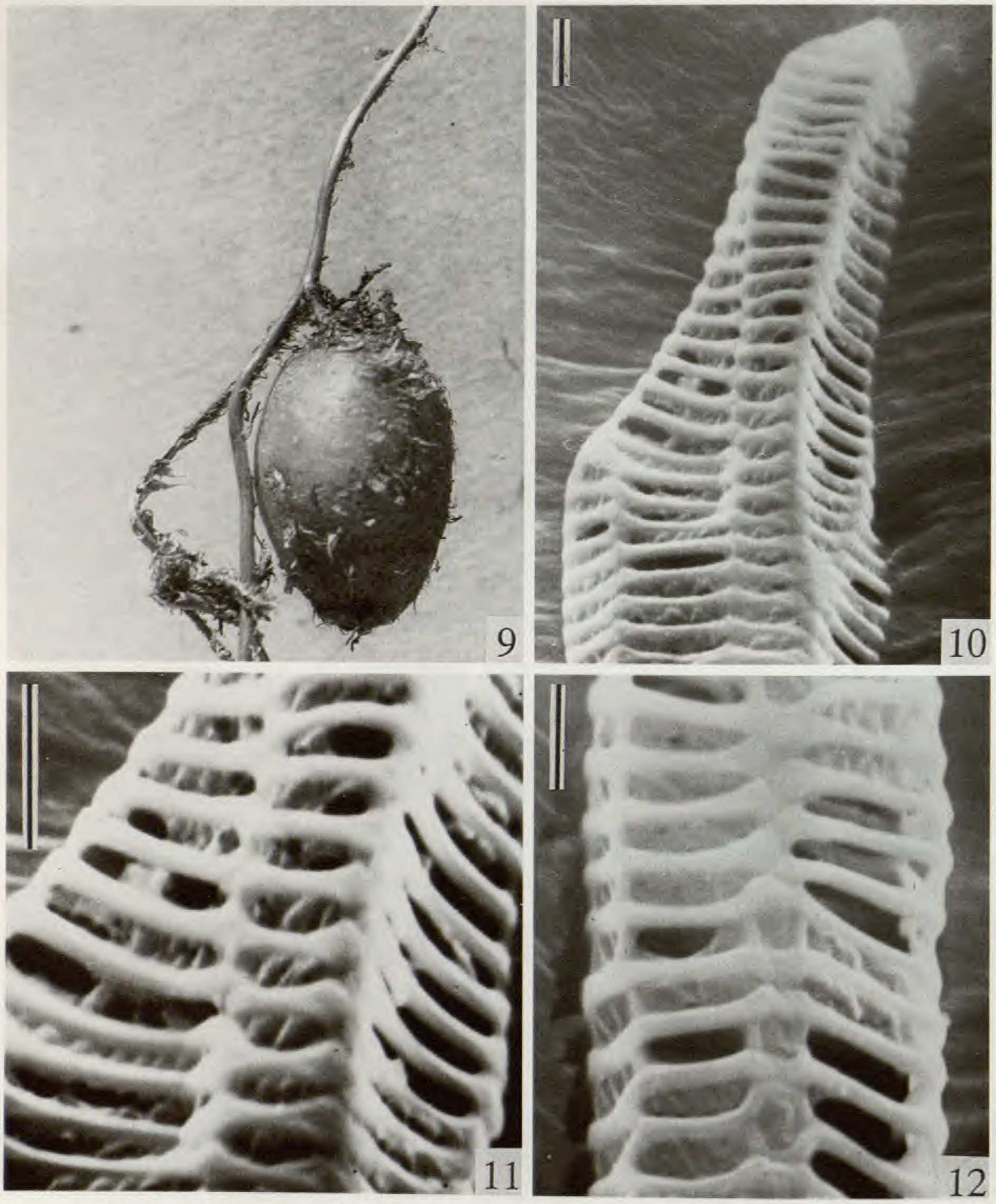
Tubers of *N. cordifolia* (Fig. 9) are borne on slender stolons a short distance below the substrate surface. Our preparations of these stolons consisted mostly of parenchyma cells in which we observed very little starch. Portions of a few tracheary elements (Figs. 10–12) were present in these preparations, however. The cell tip in Fig. 10 does not possess well-defined perforation plates. Three facets from this tip, enlarged in Fig. 11, have striate pit membranes. The walls in Fig. 11 also have some gaps in pit membranes. Some of these gaps may be artifacts, in our opinion. In Fig. 12, facets from the lateral wall of a tracheary element are illustrated. These facets bear striate pit membranes but also some membrane-free pits. Some of these we interpret as perforations.

Discussion and Conclusions

Vessel elements are clearly present in the roots, rhizomes, and stolons of *N. exaltata*. Tracheary elements of the tubers of *N. cordifolia* are not so easily identified as vessels, although some perforations were identified; tracheids as well as vessels may be present in the tubers. The presence in tubers of tracheary elements with poorly developed perforation plates correlates with the probable water storage function of the tubers. Tracheary elements adapted to rapid conductive rates are less likely to be found in tracheary elements in storage organs than in organs in which more rapid conductive rates probably occur (Schneider and Carlquist, 1998b).

Nephrolepis exaltata occurs in a variety of habitats, but some of these (including the locality at which the rhizomes, stolons, and roots were collected) have marked fluctuation in water availability. Lellinger (1985) characterized many habitats of *N. exaltata* and *N. cordifolia* as epipetric or epiphytic. Similarly rhizomatous ferns of such habitats, notably *Phlebodium* (Schneider and Carlquist, 1997), have vessel elements in which perforation plates are clearly present. Like *Phlebodium,* the perforation plates of *Nephrolepis* do not differ from lateral walls except in absence of pit membranes. Such vessel elements contrast with vessel elements of *Woodsia* species that grow in habitats with marked fluctuation in temperatures and water availability, such as *W. scopulina* (Schneider and Carlquist, 1998a) and *W. ilvensis* (Carlquist and Schneider, 1998a). In the *Woodsia* vessel elements, perforation plates have few bars and wide perforations, in contrast with lateral walls, in which the pits are narrower and shorter than perforations.

Perforation plates on lateral walls as well as end walls are present in vessel



FIGS. 9–12. Tuber (9) of *Nephrolepis cordifolia* and tracheary elements (10–12) from maceration of a tuber of *N. cordifolia*. 9) Habit of tuber, attached to stolon. 10) Tip of tracheary element, showing several facets. 11) Enlarged portion of the tracheary element shown in Fig. 10; most pits contain pit membranes, and perforation plates are either absent or poorly developed. 12) Central portion of tracheary elements, with pit membranes absent in some pits but present in most. Fig. 9, ×1; scale bars in Figs. 10–12 = 5 μm.

elements of *N. exaltata*. Perforation plates on lateral walls have been reported in a number of ferns, such as *Pteridium* (Carlquist and Schneider, 1997a) and *Phlebodium* (Schneider and Carlquist, 1997). Lateral perforation plates occur in ferns of seasonally dry habitats in which numerous vessel elements in contact with each other occur in the vascular strands.

Nephrolepis appears among more highly derived ferns in cladograms, whether based on macromorphology (Smith, 1995) or molecular evidence (Pryer et al., 1995). The vessels of *Nephrolepis* are not as specialized as those of *Pteridium, Astrolepis,* or *Woodsia* (which occupy positions of moderate to high specialization in the references just cited). Degree of specialization in perforation plates does not correlate with phylogenetic position as much as it depends on ecological and physiological factors.

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